Synaptic effects of Munc18-1 alternative splicing and protein modifications

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Summary

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The human brain is able to provide the computational power required to execute cognitive functions like perception, memory and problem solving. For this purpose, the brain consists of an extensive network of nerve cells, called neurons, which contact each other via so-called synapses. Information is passed from neuron to neuron (synaptic transmission) via the release of neurotransmitter stored in synaptic vesicles at the presynaptic site of the sending neuron. These synaptic vesicles are recruited to and docked at specialized sites on the cell membrane. There they are converted into fusion competent vesicles by a process called priming, making the vesicles ready to fuse upon arrival of an electrical trigger, the action potential. Once released these neurotransmitters will bridge the physical gap between neurons and activate receptors at the postsynaptic site of the receiving neuron. The strength of a synaptic contact largely depends on the number of primed vesicles (the size of the Readily Releasable Pool (RRP)) and the probability that such a vesicle will be released upon stimulation. Synaptic vesicle release is a tightly controlled process and modulation of this process allows for fast adaptations in synaptic strength.

Synaptic vesicle release is executed by specialized protein machinery, with at its core the SNARE proteins who drive membrane fusion, and other key proteins like Munc18. The aim of this thesis was to study different aspects of Munc18-1 function and regulation. For this purpose, we conducted a structure-function analysis of Munc18-1 in mammalian synapses using a variety of genetic mutants. To dissect their precise contribution, these mutants were expressed in neuronal cultures of *munc18-1* null mice which lack endogenous Munc18-1, rendering the introduced mutant the sole Munc18-1 variant present. Synaptic transmission was subsequently assessed using patch-clamp electrophysiology in a well-defined model system, namely neurons grown in isolation on islands of glia cells, so-called

autaptic neurons. These neurons synapse abundantly onto themselves, allowing the assessment of different physiological parameters without the complexity of a neuronal network.

The *munc18-1* gene encodes two closely related splice-variants, Munc18-1b and Munc18-1a, which differ at their far C-terminus. **Chapter 2** investigates the effect of alternative splicing of Munc18-1 on synaptic transmission. We found that expression of different splice variants had little effect on basal synaptic transmission, but that Munc18-1b expressing neurons showed less short-term depression during high frequency stimulation trains. These results suggest that Munc18-1b might be superior in sustaining synchronous release during intense stimulation. We therefore hypothesize that alternative splicing of Munc18-1 regulates presynaptic short-term plasticity. Since CaMKII inhibition had a mild effect on recovery from depression in Munc18-1a, but not Munc18-1b, the difference between the splice variants might rely on a CaMKII-dependent mechanism.

In **chapter 3** we identified Munc18-1 as a novel target for n-Src, a neuronal member of the Src family tyrosine kinases. The functional importance of this alleged post-translational modification was assessed by interfering with the main phosphorylation site on Munc18-1, Y473. This resulted in severe defects in basal synaptic transmission resulting from a reduction in the number and release probability of primed vesicles. High frequency stimulation temporarily restored priming and augmented EPSC sizes, indicating that an activity-dependent priming component was still intact. It is tempting to speculate that tyrosine phosphorylation of Munc18-1 might be a novel and potent regulatory mechanism to determine synaptic strength.

In **chapter 4** we combine quantitative biochemistry with synapse physiology and electron microscopy to address the role of Munc18-1 binding to the N-peptide of the SNARE protein Syntaxin1 in docking, priming and fusion. Since interfering with this binding mode did not hinder synaptic transmission, we argue that the N-peptide interaction mode is not essential in synaptic vesicle release. In addition, these mutants allowed us to dissect Munc18-1's function on assembled SNARE complexes and more upstream functions since they showed no detectable affinity for assembled SNARE complexes. We propose a model in which Munc18-1 assists primarily in setting up SNARE-complexes, while downstream association with fusion complexes is dispensable.

Chapter 5 extends the functional analysis of previously identified Munc18-1 constructs generated by random transposon (jumping gene) mutagenesis. The effect of a transposon insert in domain 3a at site L307 was assessed on synaptic transmission, thereby testing the involvement of this domain in synaptic vesicle release. Neurons expressing this mutant displayed reduced spontaneous synaptic vesicle release, hinting at a role for domain 3a in regulating vesicular release probability at resting conditions. In addition, these neurons displayed reduced asynchronous release and delayed recovery after stimulation. These results hint at a modest decrease in the priming capacity of Munc18-1, although the reduced Munc18-1 levels observed in this mutant might contribute to the priming defect.

Modifications of Munc18-1 thus generate diverse effects on synaptic transmission, indicating that Munc18-1 is involved in various steps of synaptic vesicle release. Different aspects of Munc18-1 function seem to be regulated by different protein-protein interactions, alternative splicing and post-translational modifications. **Chapter 6** discusses the role of Munc18-1 in several features of synaptic transmission, including priming, asynchronous release and vesicular release probability. One paragraph is dedicated to protein phosphorylation as a potent and diverse way to regulate Munc18-1 function, in which we hypothesize that several kinases converge on Munc18 on different timescales to regulate presynaptic strength. More research will be required to address the effect of simultaneous phosphorylation at multiple sites, and to identify events upstream of putative Munc18-1 phosphorylation at site Y473.

Taken together, we propose a model in which Munc18-1 is a major player of the synaptic vesicle release machinery steering the release machinery towards N-terminal SNARE assembly, thereby regulating docking, priming and release probability of synaptic vesicles.